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The Use of *Megamelus scutellaris* Berg in the Southern United States as a Biocontrol Agent of Waterhyacinth (*Eichhornia crassipes* (Mart.))

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PURPOSE: This document summarizes the development of rearing techniques and methodology for field releases of *Megamelus scutellaris* Berg (Hemiptera: Delphacidae; waterhyacinth planthopper) during the period 2010-2012. This work was accomplished through a collaborative effort that involved the US Army Corps of Engineers - Engineer Research and Development Center (USACE-ERDC) and the Louisiana State University (LSU) AgCenter. Over 3 years of adaptive rearing techniques, more than 13,875 *M. scutellaris* were released at field sites in Texas and Louisiana. In addition, because of questions regarding temperature tolerance and related mortality of various life stages of *M. scutellaris*, a study was undertaken to examine temperature differences in key internal tissues of the waterhyacinth plant and within the canopy in relationship to changes in water temperature. To the authors' knowledge, this is the first published information regarding the influence of water and air temperature on canopy and internal plant temperature in waterhyacinth.

BACKGROUND: The floating invasive plant species waterhyacinth (*Eichhornia crassipes* Mart. Solms) was first introduced into the United States in Louisiana during the International Cotton Exposition (Center 2004) in 1884. Since its primary introduction, the adventive range of waterhyacinth has spread to include the southern and western United States and disjunct northern populations (US Department of Agriculture/Natural Resources Conservation Service (USDA/NRCS) 2012). Waterhyacinth is capable of rapid growth and can quickly cover the water surface to shade out water bodies, thus reducing light penetration to algae and submersed plants, thereby lowering dissolved oxygen levels. In addition, mats of waterhyacinth have been shown to increase mosquito breeding habitat and obstruct waterways (Center 2004). The annual cost to the US Army Corps of Engineers alone for managing waterhyacinth in Louisiana currently exceeds $4 million.[3] Primary control methods include the use of herbicides and release of insect biological control agents.

Biological control of waterhyacinth has been attempted since the 1970's, with Florida releases of two weevil species, *Neochetina bruchi* Hustache and *N. eichhorniae* Warner (Coleoptera: Curculionidae), and a moth species, *Niphograpta albiguttalis* Warren (Lepidoptera: Crambidae) (Center 2004). Establishment of *Neochetina* spp. has been widespread, and the biocontrol agents are now ubiquitous in the Southeast. More recently, South American surveys by the USDA Agricultural Research Service

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[3] Personal Communication. October 2010. Michael Saucier, US Army Engineer District, New Orleans.

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(USDA/ARS) have focused on *Megamelus scutellaris* Berg, a planthopper associated with waterhyacinth in the majority of its native range (Sosa et al. 2004).

Megamelus scutellaris is a small delphacid planthopper that feeds on the phloem of waterhyacinth plants by piercing vascular tissue (Figure 1). Adult *M. scutellaris* mate at the base of petioles and near leaf-petiole junctions. Eggs are generally laid by insertion near leaf-petiole junctions though other areas on the petiole can be used.

Figure 1. *M. scutellaris* nymph on a leaf of waterhyacinth.

Megamelus scutellaris were first imported into US quarantine from Argentina in April 2006. After rigorous host-specificity testing and review by the Technical Advisory Group (TAG) for Biological Control of Weeds, approval to release *M. scutellaris* in the United States was granted in February 2010.[1] The first releases of *M. scutellaris* took place in Florida in 2010 by USDA/ARS researchers. Although tentative field establishment has been reported in Florida and California,[2, 3] there has been some question as to whether extreme summer temperatures commonly encountered in Texas and Louisiana would prevent permanent establishment of *M. scutellaris* in these areas. Such reasoning is based on both seemingly poor establishment success and an unpublished report by South African researchers that indicated high mortality of *M. scutellaris* first nymphal stages at temperatures as low as 29 ºC.

The objectives of this work were to: 1) continue rearing efforts to allow maximum colony production with subsequent field release of large numbers of *M. scutellaris,* and 2) determine what

[1] Personal Communication. April 2010. Dr. Al Cofrancesco, Technical Director, Environmental Laboratory, US Army Engineer Research and Development Center, Vicksburg, MS.
[2] Personal Communication. November 2013. Phil Tipping, US Department of Agriculture/Agricultural Research Service, Davie, FL.
[3] Personal Communication. October 2013. Patrick Moran, US Department of Agriculture/Agricultural Research Service, Albany, CA.

effects air and water temperatures have on temperatures within the waterhyacinth canopy and within waterhyacinth plant tissues relative to their position within the canopy.

MATERIALS AND METHODS

ERDC rearing activities. Populations of *M. scutellaris* were obtained from USDA/ARS in May 2010 (~700 individuals), February 2011 (~300 individuals), and again in May 2012 (~600 individuals). Upon receipt, waterhyacinth planthoppers were placed on healthy, green waterhyacinth plants growing in greenhouse cultures under natural light. Waterhyacinth planthoppers were maintained on waterhyacinth cultured in two shallow 1.2-m x 2.4-m x 0.25-m tanks (Figure 2). Fertilizer (0.0375 ml/L of a 50 g/L Peters®20-20-20 (Israeli Chemicals Ltd. (ICL), Tel Aviv, Israel) stock solution and 0.10 ml/L of a 20 g/L 6% iron chelate stock solution) was added to the water as needed to maintain vigorously growing waterhyacinth.

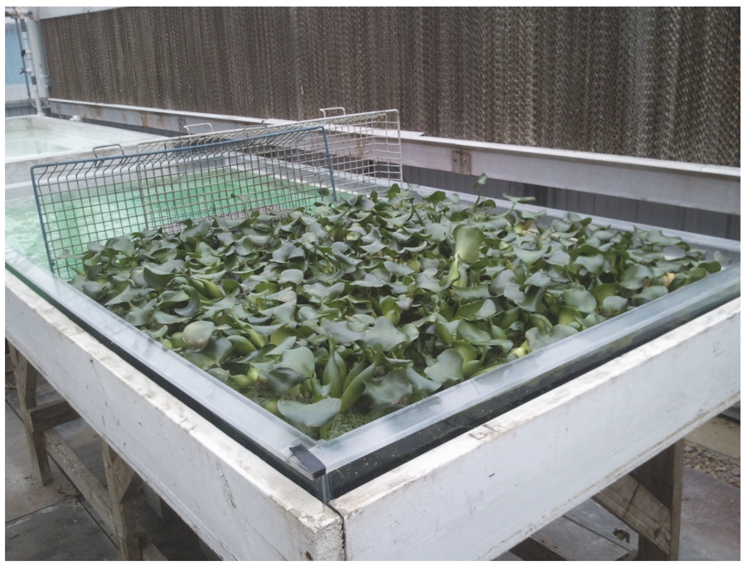

Figure 2. Waterhyacinth cultures maintained at USACE-ERDC, Vicksburg, Mississippi. After plants were mature and canopy-forming, *Megamelus scutellaris* individuals were placed on the plants from shipments obtained from USDA/ARS.

No formal counting procedures for waterhyacinth planthopper population sizes were applied during culture development. However, waterhyacinth planthopper populations were estimated based upon presence of multiple life stages (adult and various nymph instars). This information was combined with visual assessments of general condition of the waterhyacinth plants (e.g., showing signs of damage or decline) to determine when to thin cultures for either field release or removal of insects for stocking into refresh cultures (healthy plants). Individuals were moved by aspirating them into a plastic container using a vacuum pump and gently shaking the container over new plants. In general, waterhyacinth planthoppers were moved to new material in increments of 100 individuals.

In 2011 and 2012, to combat high temperatures experienced in the greenhouse and infestations of southern red mite (Trombidiformes: Tetranychidae, *Oligonychus ilicis*; Figure 3**)**, a water misting

system was installed. Misters were placed just above the plants and attached to timers set at specific intervals (i.e., every 2 hr for 5 minutes) of misting throughout the day. In addition, the predatory mite, *Amblyseius fallacis* (Parasitiformes: Phytoseiidae), was introduced to help control red mite populations.

Figure 3. *Oligonychusilicis* McGregor (southern red mite) infestation of waterhyacinth cultures in 2011.

In 2012, rearing of *M. scutellaris* was slowed by unexpected changes in city-delivered water. The pH of the water used in plant culture tanks, even after being processed using a deionizing system, was over 9.0 and greatly reduced waterhyacinth growth. This represents a 2-point increase in pH over the pH of the water typically used in the greenhouse in the past. This pH is also outside the range for optimal waterhyacinth growth (El-Gendy et al. 2004). Unfortunately, this change in water quality was only recognized when it became difficult to grow healthy waterhyacinth, and an *M. scutellaris* colony could not be initiated until early summer when healthy and actively growing waterhyacinth cultures could be established. This effectively eliminated any releases from the USACE-ERDC cultures for 2012.

LSU AgCenter rearing activities. *Megamelus scutellaris* were produced in a greenhouse during 2011and 2012 in 568-L structural foam tanks (1.5 m x 1 m x 0.6 m) and maintained at a temperature of 23-29 ˚C with natural daylight (Figure 4). Reverse osmosis (RO) water was used in the tanks with periodic application of nutrients. Waterhyacinth was treated with the addition directly to the water of Miracle-Gro® (ScottsMiracle-Gro Company, Marysville, Ohio) 36-6-6 water-soluble fertilizer as well as 200 ml of chelated iron (active ingredient - iron ligosulfonate, Fe_2O_3 content - 4.7% by wt., 0.15 kg/3.8 l of iron). Liquid chelated iron was also added to the tanks at a rate of 0.2 g/L when plants displayed symptoms of iron deficiency (i.e., yellow-streaked leaf blades). The culture of waterhyacinth was initiated with field plants collected from roadside canals near Gramercy, Louisiana (2010 and 2011) and Krotz Springs, Louisiana (2012). Since waterhyacinth planthopper establishment may be

hindered by existing herbivores, plants were treated with a tank mix of the insecticide Karate® (Lambda-cyhalothrin – 22.8% a. i., Syngenta, Wilmington, Delaware) and ammonium sulfate (NH4+) when first brought in the greenhouse. This was done to fertilize plants and kill *Neochetina* spp. weevils in an effort to prevent competitive interactions between the weevils and waterhyacinth planthoppers. Karate® was applied using the recommended application rate for rice water weevils (*Lissorhoptrus oryzophilus* Kuschel) (13.6 g a.i./acre of water).

Figure 4. Culture tanks used at LSU AgCenter for rearing *Megamelus scutellaris.*

Starter populations of *M. scutellaris* were obtained from USDA/ARS in February 2011(~400 individuals) and again in February 2012 (~200 individuals). Upon receipt, *M. scutellaris* individuals were placed on healthy, green waterhyacinth plants growing in greenhouse cultures.

The *M. scutellaris* colony was monitored on a weekly basis to follow age distribution of waterhyacinth planthoppers and presence of southern red mites. Southern red mites were a periodic problem in the greenhouses and populations can increase quickly; therefore, frequent inspection was required. As needed, during 2012, spray applications were made with the Kelthane® 50 WSP (Dicofol50% a.i., Rohm Haas, Philadelphia, Pennsylvania) at a rate of 0.7g wettable powder/L of water. This application rate was selected after preliminary trials in June indicated that the application of miticide had no effect on *M. scutellaris.* Misting at frequent intervals (10 min/2 hr) helped maintain temperatures within the desired range (23-30 °C) and reduced mite populations to more acceptable levels. Summer temperatures were recorded, but no spikes above 32^{o} C were noted in the greenhouse other than one

episode in June. The length of misting was increased to 30 min/2 hr and held the temperature to just 3-4 spikes over 32° C with a mean of 28 °C. Waterhyacinth planthoppers appeared healthy in July, with developing immatures present in the cultures at that time.

Release and establishment techniques. In 2010, releases were made with individuals obtained from colonies maintained at USACE-ERDC. Individuals were harvested from the colony by using an aspirator attached to a small vacuum pump. In other cases, individual leaves containing high numbers of individuals (visually estimated at more than 50 per leaf) were carefully removed from the plant and subsequently shipped or hand carried to the release sites. For transport, waterhyacinth planthoppers were placed into containers lined with moistened paper towels and a few waterhyacinth leaves. Releases consisted of a mixture of late instars or adults whenever possible.

In 2011, releases were made from colonies established at USACE-ERDC as well as from the LSU AgCenter. Similar release techniques were used as outlined for 2010. For the Lake St. Joseph, Louisiana (Table 1) site near Newellton, Mississippi, individuals were released into a 1-m^3 polyvinyl chloride (PVC) pipe frame, covered in 500-µm insect mesh. On one side of the cage, mesh was affixed to the PVC with hook and loop fasteners to allow easy access to the plants inside. A HOBO® (Onset, Cape Cod, Massachusetts, USA) temperature logger was hung from the top frame by string and placed among the waterhyacinth plants at the water surface to record canopy air temperature. Temperatures were recorded every 30 minutes beginning 2 August 2011 and continued through 11 May 2012. The entire apparatus (cage and temperature logger) was placed on the waterhyacinth mat with the mat producing enough buoyancy to keep the cage afloat.

In 2012, 5-m^2 release plots in southern Louisiana were first sprayed with Karate® (13.6 g a.i./acre)[1] and ammonium sulfate (NH4+) prior to *M. scutellaris* releases to control waterhyacinth weevil populations and stimulate plant growth. A dense stand of waterhyacinth plants (~ 20 plants/m^2) covered the surface of each plot at the time of pesticide application. Each location received a screened cage approximately 1.5 m^2 x 1 m^2 with the interior of the cages accessible through a long zipper on the side of the cage.

Temperature study. Waterhyacinth plants used for the temperature experiment were grown in three separate polyethylene tanks (76.2 cm x 88.9 cm x 82.3 cm), each fitted with a separate liquid circulator (RemcorModel CFF-500, Glendale Heights, Illinois). Water temperatures were held at 18 °C, 26 °C, and 33 °C under greenhouse conditions during mid-July 2012 at USACE-ERDC. The tanks were filled with deionized water and fertilized as described previously. Waterhyacinth plants were placed into each tank to form a canopy that covered a minimum of 80% of the water surface. Temperature readings were obtained after the plants were allowed to acclimate for a minimum of 7 days. Temperature readings were measured using k-type beaded thermocouples in conjunction with a series of Reed Model SD-947 four-channel digital thermometers (Reed Instruments, Inc., Texas). Temperatures were recorded at 5-minute intervals for a period of approximately 4 days, beginning on 16 July 2012. Ambient air temperature was recorded, along with temperatures at the upper and lower portions of the plant canopy, within the petiole tissues at the junction of the leaf lamina and petiole, and within the lower petiole about 2 cm above the water surface. The thermocouple used to monitor ambient air

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[1] Karate® is labeled for use on flooded rice (*Oryza sativa*) and wild rice (*Zizania* spp.) to control several pest species, including rice weevils.

temperatures was housed in a shielded box equipped with slanted open sides. This allowed air to circulate around the thermocouple in an effort to prevent excess heating due to solar radiation. Temperatures within the plant tissues were obtained from a single, randomly selected plant. Thermocouples were inserted into plant tissues about midway into the upper and lower petioles of the plant.

RESULTS AND DISCUSSION

Rearing. Initial colony setup and rearing at ERDC were considered successful in 2010. From the initial release of nearly 700 individuals, the colony reproduced and expanded to infest all plants and most plant parts within the waterhyacinth culture, providing enough individuals to start new colonies and make field releases. Labor to maintain a greenhouse colony was relatively minimal compared to previous projects rearing the salvinia weevil (Harms et al. 2009, Knutson and Nachtrieb 2012). Maintenance included replenishing water and adding fertilizer when plants appeared to be nutrient stressed. A series of releases were made early during the growing season (May and June), but after this time the colony numbers declined to where less than one individual could be found every five plants. Initially, it was believed that the colony was depleted because of the high numbers of individuals removed for release at field sites. In response, releases were discontinued to permit recovery of greenhouse colonies. However, the expected increases never occurred. By the end of September 2010, the colonies had declined to the point where no individuals could be detected. Similar declines were experienced at colonies maintained at the USDA/ARS laboratory in Ft. Lauderdale, Florida. Reasons for these declines were unknown, but work by South African researchers (unpublished report) has suggested thermal limits for this strain of *M. scutellaris* occur at temperatures below those experienced in the greenhouse. The South African researchers reported that mortality in the first nymphal instar increased substantially when temperatures were over 29 ^{o}C.

In 2011, efforts were made to increase colony numbers earlier in the growing season so field releases could commence before higher temperatures began impacting population numbers in both greenhouse and field locations. In addition, a colony was initiated at the LSU AgCenter in an effort to increase numbers of individuals as well as minimize the colony. Personnel at both facilities were able to achieve this goal, with field releases occurring before the end of June in 2011. Southern red mites continued to be problematic at both locations even with the introduction of predatory mites and frequent misting.

In 2012, water quality issues and high temperatures plagued early-in-the-season rearing efforts at both facilities, but a colony was established in October 2012 in a temperature-controlled environmental chamber at USACE-ERDC. This chamber will be kept at temperatures below the reported lethal limits to determine if temperature effects can be moderated. Information on the results of colony growth at lower temperatures will be provided at a later date.

Release and establishment. Since July 2010, over 13,000 individuals have been produced by the two rearing facilities and released in two states at seven locations (Table 1). This includes six field locations as well as at the Lewisville Aquatic Ecology Research Facility (LAERF), Lewisville, Texas, where a third greenhouse culture is being established. Field release sites were chosen based on several defining characteristics. These included minimal impact due to human activities, ease of access, and the presence of healthy expanding populations of waterhyacinth. A large number of individuals (~ 1000) were sent to the LAERF with the purpose of establishing an additional colony outdoors, thus allowing

for a backup source of *M. scutellaris*, in the event of colony decline in the original rearing and release locations. Unfortunately, the colony failed to establish at the LAERF, apparently due to lethal high temperatures.

Table 1. Numbers of waterhyacinth planthopper released in Louisiana and Texas beginning in 2010.

Site	Coordinates	Date Range	~Numbers Released
Gramercy, LA	30°11'4.61"N	7/7/2010 10/10/2010	600
	90°49'15.38"W		
Keyhole Lake, Bonnet Carré Spillway, LA (Open release)	32°20'35.01"N	10/10/2010	200
	90°59'8.73"W		
Delta, LA (Open release)	32°20'35.01"N	8/5/2010	400
	32°20'35.01"N		
Lewisville, TX (In greenhouse)	33° 4'6.90"N	7/20/2010 8/4/2010	1,000
	96°57'11.41"W		
		TOTAL 2010	2,100
Gramercy, LA (Open release)	30°11.087'N	5/2011 6/2011	4,600
	90°49.261'W		
Paradis, LA (Open release)	29°53.021'N	5/2011 6/2011	4,600
	90°28.642'W		
Lake St. Joseph, LA (Single cage)	31°54'10.43"N	9/15/2011	200
	91°14'29.99"W		
		TOTAL 2011	9,400
Gramercy, LA (Two cages in close proximity)	30°11'3.48" N	6/12/2012	625 (Range 500-750)
	90°48'55.45"W		
Alligator Bayou, LA (Two cages in close proximity)	30°18'31.46"N	6/12/2012	625 (Range 500-750)
	91°0'59.64"W		
Paradis, LA (Single cage)	29°53'47.86"N	6/13/2012 6/29/2012	1,125 (Range 500-1,000)
	90°28'3.95"W		
		TOTAL 2012	**2,375**
		Overall Total	**13,875**

Despite the number of releases and release techniques attempted, establishment could not be confirmed at any Louisiana location. Reasons for this are not entirely understood but temperatures over the lethal limit could have played an important role. In addition, mitigating circumstances at several of the sites may have contributed to establishment failure.

In 2010, 200 waterhyacinth planthoppers were released at a borrow pit in Delta, Louisiana. However, this site was eliminated from consideration for additional releases in 2011 after historic levels of flooding from the Mississippi River flushed out all the waterhyacinth (Figure 5).

Additionally, due to that flooding, evidence of establishment from the 2010 release could not be found. The site was examined again in 2012 to determine its suitability as a release site, but waterhyacinth had not recovered to an extent that warranted further releases at that time.

Figure 5. Site near Delta, Louisiana showing increases in water level during historical flooding occurring on the Mississippi River during the spring of 2011. Flooding flushed out all the waterhyacinth with no recovery of the infestation even into 2012.

In 2011, additional waterhyacinth planthoppers were received from USDA/ARS and greenhouse colonies were reestablished at ERDC. Although the colonies were robust enough to support early field releases, flooding at potential release sites meant releases could not be accomplished early in the growing season when temperatures were typically below lethal limits. By August 2011, the greenhouse colony again appeared to be in decline, triggering a single field release of 110 individuals into a screen cage at Lake St. Joseph, Louisiana. In May 2012, Lake St. Joseph was revisited to retrieve the release cage, but the presence of *M. scutellaris* could not be confirmed. Small cast skins, which appeared to resemble *M. scutellaris*, were observed on waterhyacinth leaves and a single small, immature planthopper was collected but was damaged beyond identification during transit back to the laboratory. A subsequent visit to the site toward the end of May revealed that the site had been treated with herbicides, which reduced the waterhyacinth to minimal levels and eliminated any chance of confirming establishment and continuing releases.

Examples of extreme mitigating circumstances precluding establishment occurred at several other sites. On 18 July 2012, following a June release, the two cages at Alligator Bayou were examined and waterhyacinth planthoppers were observed in both cages with the population in cage #2 appearing to support more insects than were released. On 1 August 2012, waterhyacinth planthoppers were observed in cages at both Gramercy and Alligator Bayou; populations appeared lower at Alligator Bayou. Spiders were observed in cages at Alligator Bayou and may have contributed to poor waterhyacinth

planthopper establishment. On 15 August 2012, waterhyacinth planthoppers at release sites in Gramercy and Paradis were not observed.

A visit planned for September 2012 to check waterhyacinth planthopper release sites at Alligator Bayou, Gramercy, and Paradis was canceled because cages were inaccessible due to extreme flooding from Hurricane Isaac. The water level was 1.8 m above normal at Gramercy, and cages were submersed on one end because the anchor rope was too short, thus not allowing the cage to rise with increasing water levels. The Gramercy sites had approximately 3 m of floodwaters and the landowner indicated that the site would not be accessible until early November.

Temperature study. Apparent high temperature impacts and an unpublished South African research report indicated high mortality of first instar nymphs at temperatures approaching 30 ^{o}C. Therefore, experiments were conducted to compare temperatures within the waterhyacinth canopy and within certain tissues of the plant (i.e., those most likely to house waterhyacinth planthopper eggs) to water and air temperatures.

Three different water temperature treatments (18^{o}, 26^{o}, and 33 ^{o}C) were maintained throughout the experiment, with recorded deviation of $\pm$ 2^{o} C (Figure 6). Even with this variation, distinct high, medium, and low water temperature ranges were maintained throughout the entire 4-day experiment.

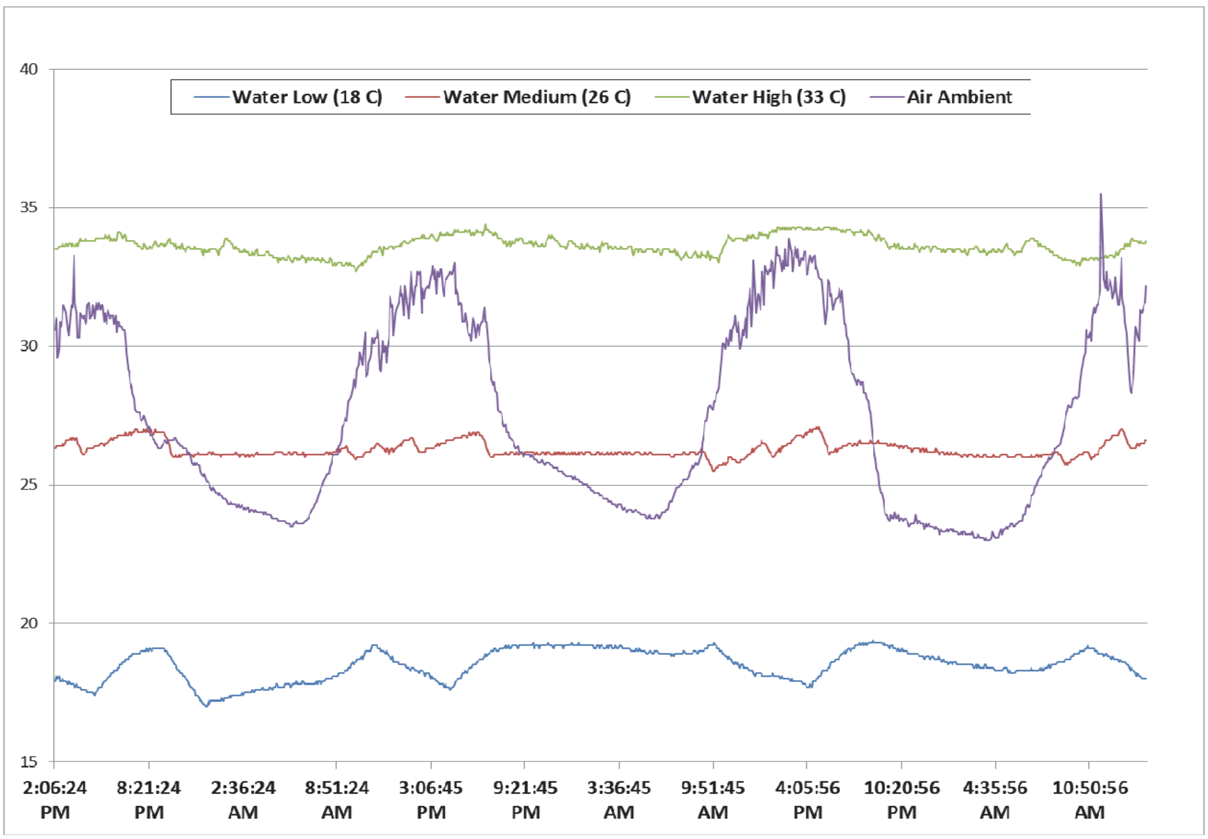

Figure 6. Ambient air and water bath temperatures maintained during a July 2012 temperature study.

It was difficult to discern distinct trends or relationships when examining the data in raw form. Temperatures associated with the upper canopy and upper petiole appeared to be more closely related to air temperature than water temperature. As air temperature increased, temperature in the upper canopy and upper petiole appeared to increase as well. These relationships become clearer when examining the data using multiple regression techniques. This relationship is significant for both air and water temperature when performing a multiple regression using either upper petiole or upper canopy as dependent variables against both air and water temperature (upper canopy: $F(2, 2610) = 34{,}404.78$, $p < 0.05$, adjusted $R^2 = 0.96$ and upper petiole: $F(2, 2619) = 25{,}764.86$, $p < 0.05$, adjusted $R^2 = 0.95$; see Figure 7). The use of both air and water temperature in the model explains over 96% of the variation in upper canopy temperature. Also, while more than 95% of the variation in upper petiole temperature can be explained by changes in air and water temperature, air temperature appears to be the more important driver of upper canopy and upper petiole temperature. This is shown visually in Figure 7 where the slope of the plane is nearly 40% greater for increases in air temperature than that shown for similar increases in water temperature. Interestingly, temperature in the upper canopy, while generally lower than air temperature, shows greater difference with increasing air temperature according to the regression model. When air temperature is 30^{o} C and water temperature is 20 ^{o}C, the predicted upper canopy temperature is 28.5 ^{o}C; i.e., 1.5 degrees less than air temperature. When air temperature is 40 ^{o}C and water temperature is 20 ^{o}C, the predicted upper canopy temperature is 35.8 ^{o}C; i.e., 4.2 degrees less than air temperature. This represents a 2.8-fold decrease within the upper canopy temperature with a 10 ^{o} C rise in air temperature. Similar depressions in upper petiole temperature also occur, although these seem to be less extreme than that observed for the upper canopy.

Reasons for decreased upper canopy and petiole temperature relative to increasing air temperature are unknown and were not expected. This may be related to increased evapotranspiration by the waterhyacinth plants, thereby raising relative humidity at higher daytime temperatures, thus lowering temperatures within the canopy.

Lower canopy and lower petiole temperatures were influenced by both air and water temperatures (Figure 7). This is shown by a significant multiple regression for both lower canopy and lower petiole values compared against air and water temperature (lower canopy: $F(2, 2610) = 35{,}918$, $p < 0.05$, adjusted R2 = 0.9649, lower petiole: $F(2, 2610) = 8{,}476.9$, $p < 0.05$, adjusted $R^2 = 0.8665$). As with the upper temperatures, both lower canopy and petiole temperatures explain a high proportion of variation with adjusted R^2 values exceeding 0.85. As water temperature (and, to an extent, air temperature) increased, temperature within the lower canopy and petiole increased. This can be visualized by the relatively large and similar slopes for both air and water temperatures (Figure 7). As was observed for upper canopy and petiole temperatures, depressions in temperatures within the lower canopy and petiole relative to air and water temperatures increased as air and water values rose.

The biological significance of this information is important. With the probable heat-related mortality recognized for the waterhyacinth planthopper, understanding actual exposure temperature relative to air temperature is critical. The data suggest that while air temperature may increase to thresholds where mortality is predicted to occur, actual petiole temperature can be quite a bit lower, and possibly below mortality limits. With predicted depressions in upper petiole temperatures being close to 3.5 ^{o}C lower than high air temperatures, actual mortality thresholds may not be reached until air temperatures are substantially higher. However, more research is needed. This is especially

relevant since this experiment was conducted under greenhouse conditions during only one of the hotter times of the growing season. Field-based temperature profiles should be developed to reflect a variety of different temperature ranges as well as different canopy structures. In addition, it may be important to determine humidity levels in reference to lowered temperatures within the canopy.

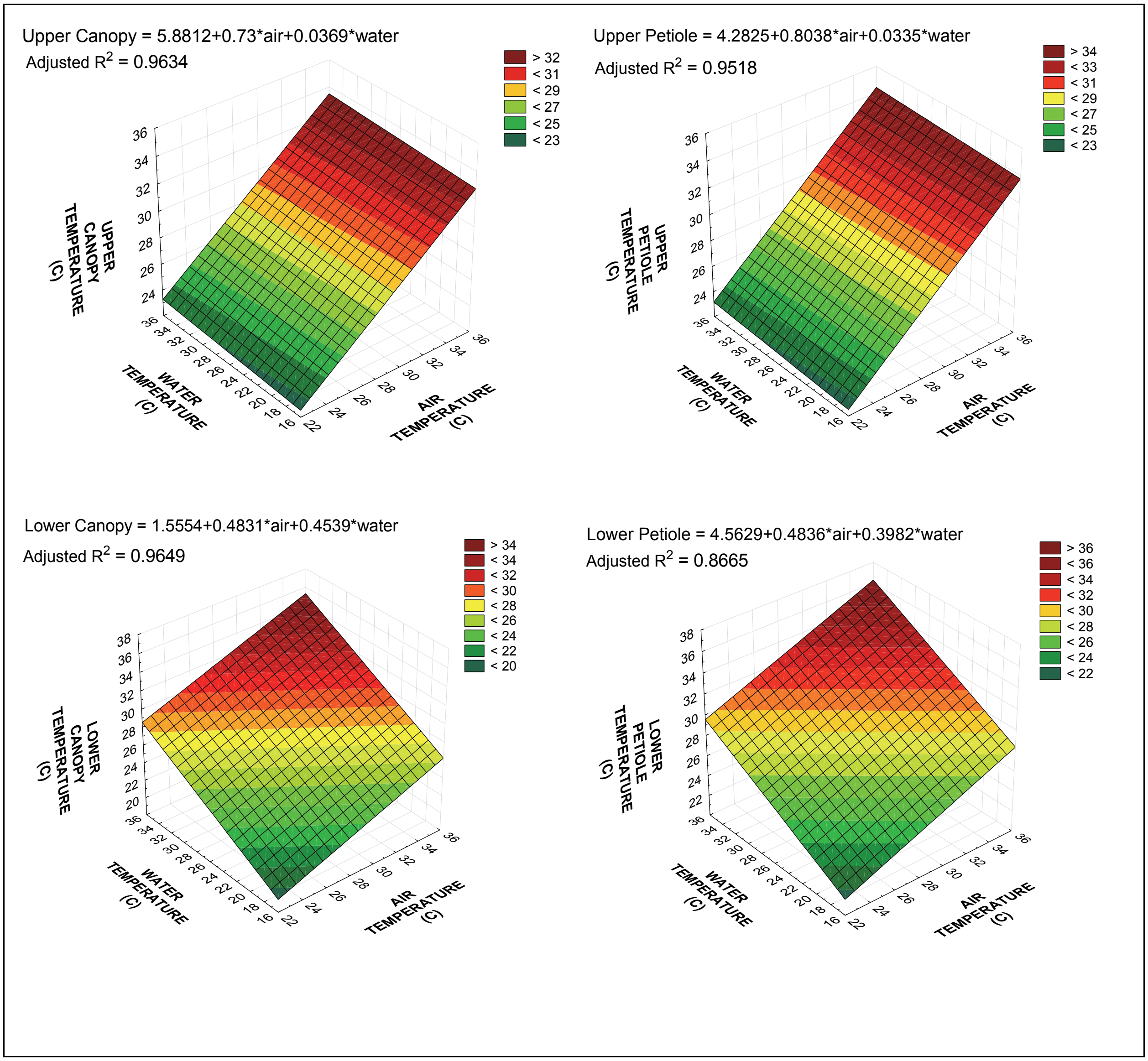

Figure 5. Predicted temperatures based on multiple regression analyses performed for upper canopy/petiole and lower canopy/petiole against air and water temperatures. All parameters in the model were significant (i.e., air and water) at $p < 0.05$. Adjusted R^2 values and the predicted model equation are depicted on the graph.

The observed behavior of *M. scutellaris* indicates some degree of thermoregulation as it relates to their movement on the plant. During the hottest part of the day and growing season, waterhyacinth planthoppers tend to remain close to the lower petiole region, presumably where temperatures are more moderated by cooler water temperatures. It has also been observed in *M. scutellaris* rearing colonies at ERDC that oviposition scars occur at various locations along the petiole, which could be a behavioral

response to increased air temperatures in the greenhouse, since presumably egg development would be affected by higher temperatures.

Future directions. Because of apparent temperature-related population declines in rearing colonies from multiple agencies, attempts are underway to establish a new colony of a high temperature-tolerant strain of *M. scutellaris* in the USDA/ARS Florida laboratory from individuals collected in Argentina. If laboratory colonies are successfully established from the new sources, then future releases could be initiated earlier in the growing season when temperatures are cooler to offer a better chance at field establishment.

Another avenue may be to begin experiments with the goal of selecting temperature-tolerant individuals of the current strain. This could potentially be accomplished with temperature-controlled environmental chambers over many successive generations of reproducing waterhyacinth planthoppers. This selection process has the potential to produce agents that are more tolerant of higher temperatures typically found in association with the US distribution of waterhyacinth.

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